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CROSS TOLERANCE BETWEEN LSD-25 AND PSILOCYBIN.

Recently it has been shown (Hofmann et al, 1958a; Isbell, 1959) that O-Phosphoryl-4-hydroxy-N-dimethyl tryptamine (hereafter referred to as psilocybin), a compound isolated from certain species of mushrooms (Hofmann et al, 1958b) which are used ceremonially by Mexican Indians (Wasson and Wasson, 1957), has psychotomimetic properties very similar to those of LSD-25. The close resemblance of the patterns of symptoms induced by LSD and psilocybin suggested that these drugs cause a psychosis by some common biochemical or physiological mechanism. Since the effects of LSD diminish rapidly when the drug is given daily (Isbell et al, 1956), it was felt that the hypothesis that the LSD and psilocybin psychoses are identical could be tested by determining if cross-tolerance existed between the two drugs. In other words, if the degree of the reaction induced by a given dose of psilocybin was significantly less in a person made tolerant to LSD, cross tolerance would be said to exist, and, conversely, the reaction to a given dose of LSD should be reduced in a person tolerant to psilocybin. In the latter case, it is implied that tolerance to psilocybin would develop.

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METHODS

Subjects. Five male former opiate addicts who were serving sentences for violation of the U. S. narcotic laws volunteered for the experiments. Their ages varied between 25 and 35 years. All were physically healthy and none presented any evidence of the major psychoses. All had received LSD-25 in previous experiments.

General Conditions. The subjects were housed in a special ward devoted to clinical research. Temperatures, respiratory rates and blood pressures were measured three times daily after the patients had rested quietly in bed during days in which special measurements were not being made. The patients were observed by specially trained aides with long experience in detecting drug-induced changes in behavior.

Drugs. The design of the experiment is summarized in Table 1. LSD and psilocybin were given in 30 cc of cherry syrup at 8 a.m. with the patients fasting. All doses were calculated on a mcg/kg basis. Control experiments were carried out, using 1.5 mcg/kg of LSD, 150 mcg/kg of psilocybin and a placebo, before the first period of chronic intoxication and after the period of placebo medication prior to the second period of intoxication. Control tests with LSD and psilocybin were

conducted at least five days apart in order to prevent the development of tolerance. The order of administration of placebos, LSD and psilocybin was randomized in both control periods.

There were two periods of chronic drug administration -- one with LSD and one with psilocybin. The order in which the 5 patients received the drugs chronically (first or second) was randomized, with 3 receiving LSD first and 2 receiving psilocybin first. After the necessary measurements had been obtained, LSD or psilocybin was replaced with placebos for at least seven days, after which the second controls were obtained. Following this placebo period, those patients who had received LSD chronically received psilocybin chronically, and vice versa.

The doses of LSD and psilocybin on the first day of chronic intoxication were 0.25 mcg/kg and 25 mcg/kg respectively. These doses were increased by 0.25 mcg/kg (LSD) or 25 mcg/kg (psilocybin) on each succeeding day until 1.5 mcg/kg of LSD or 150 mcg/kg of psilocybin were being given on the sixth day. On the eighth or ninth day of chronic administration detailed measurements were made after administration of the drug which the patient had been receiving chronically. This served as a

test of "direct" tolerance to that drug. On the subsequent day, the patient was "challenged" with the drug (1.5 mcg of LSD or 150 mcg of psilocybin) that he had not been receiving. This served as a test of "cross" tolerance.

The patients were then placed on placebos for 7-10 days after which they were tested again with placebo, 1.5 mcg/kg of LSD, and 150 mcg/kg of psilocybin. These "second controls" were obtained to determine if tolerance had been lost.

The patients were then returned to chronic medication following the schedule described above, with those patients who received LSD in the first period of chronic administration now being given psilocybin, and vice versa. The patients were then challenged with LSD and psilocybin in the manner described above.

Observations. During each day of the control periods and the periods of chronic drug administration during which the patients were "challenged" with placebo, LSD or psilocybin the following observations were made at hourly intervals twice before and eight times after administration of the drugs: rectal temperature, pulse rate, systolic blood pressure, pupillary size, and threshold for elicitation of the kneejerk.

The methods used were those previously described (Isbell et al, 1956; Isbell et al, 1959; Isbell, 1959). In addition the patients completed, with the help of an aide, a special questionnaire at hourly intervals from 7:30 a.m. to 3:30 p.m. At these same times, general notes on behavior were written. Clinical grades of the intensity of the reaction were assigned on the basis of the system of Isbell et al (1956).

Analysis of Data. After drug administration the change in rectal temperature, pulse and respiratory rates, pupillary size, blood pressure and threshold for elicitation of the knee jerks were calculated by subtracting the average of the two pre-drug observations from the values obtained at various hours. The areas under the time-action curves for each particular measurement composed of these figures were calculated by the method of Winter and Flataker (1950), thus converting all the data on a particular subject, a particular drug and a particular day to one figure termed "degree-hours" (temperature), "beat-hours" (pulse rate), etc. The total number of positive responses on the questionnaire were counted over the entire period, eliminating answers which were also scored positively before the drug. Means and standard errors of means were calculated according to standard statistical techniques.

The differences in the various measurements after placebo, 1.5 mcg/kg of LSD, and 150 mcg/kg of psilocybin in the first and second control periods were evaluated by a t-test for paired observations (Edwards, 1946). Since none of the differences were statistically significant in the two sets of controls, they were averaged and the averages used in evaluating the presence and degree of tolerance and cross-tolerance.

The differences in the effect of LSD and psilocybin after chronic administration of LSD and psilocybin were then evaluated by the same statistical technique for differences in paired observations. Four comparisons were made: (1) response to LSD after chronic administration of LSD (direct tolerance to LSD); (2) response to psilocybin after chronic administration of LSD (cross tolerance LSD and psilocybin); (3) response to psilocybin after chronic administration of psilocybin (direct tolerance to psilocybin); and (4) response to LSD after chronic administration of psilocybin (cross tolerance psilocybin and LSD). The signs of the differences were so arranged that a minus (-) sign indicated a decrease in the measurement after chronic intoxication as compared with control, and a plus (+) sign indicated an increase.

RESULTS

The differences between the first and second controls are shown in Table 2. None of the differences are statistically significant, as indicated by the large standard errors. There did seem to be a tendency for the measurements of pulse rate, blood pressure, and threshold for the kneejerk to decrease on the second control. A slight decrease in the number of positive responses on the second control also occurred with both LSD and psilocybin. These decreases may possibly indicate that some degree of residual tolerance and cross tolerance was present even after a week's time.

The differences in the responses to LSD and psilocybin after chronic administration of either drug are shown in Table 3. The first column shows the response to LSD after chronic administration of LSD, and reflects direct tolerance to LSD. The second column shows the response to psilocybin after chronic treatment with LSD, and reflects "cross" tolerance between LSD and psilocybin. The third column shows the response to LSD after chronic administration of psilocybin, and is a measure of cross tolerance between LSD and psilocybin. The last (fourth) column shows the response to psilocybin after chronic treatment with psilocybin, and is a measure of direct tolerance to psilocybin.

Inspection of the table shows that all the signs were minus signs, which suggests the presence of tolerance and cross tolerance to both drugs. In the case of direct tolerance (first column) to LSD, the changes were highly significant statistically ($P < 0.01$) on three measures (blood pressure, pupillary size, and clinical grade) and significant ($P < 0.05$) on one measure (pulse rate). In the case of direct tolerance to psilocybin (fourth column), highly significant decreases were observed in threshold for the kneejerk, and on clinical grade. A significant change in the pulse rate was also seen. In the case of cross tolerance between LSD and psilocybin, a highly significant decrease was observed in the threshold for the kneejerk, and significant decreases were seen on blood pressure, pupillary size and clinical grade. The results are not as clear in case of cross tolerance between psilocybin and LSD (column 3). Although the responses were less on all measures, the changes were significant only in the cases of the blood pressure and clinical grade.

The data leave little doubt that a great deal of direct tolerance to LSD was induced, and that, while the patients were tolerant to LSD, considerable cross tolerance to psilocybin was present. In the case of direct tolerance to psilocybin,

interpretation is not as easy. Although all the changes were in the proper direction, and, in the case of two of the measurements, highly significant, the magnitude of the decrease in the responses was less than with direct tolerance to LSD. These facts suggest that, although tolerance to psilocybin does develop, it develops more slowly or is less complete than is tolerance to LSD. For this reason, the relatively small (though consistent) decline in the response to LSD after chronic treatment with psilocybin is not surprising.

The failure to reach significance in the decrease in responses on the questionnaire was due in every case to one individual who was very sensitive to both drugs while not tolerant. He scored over 200 responses after LSD, and over 90 responses after psilocybin in both the first and second controls. The number of responses on challenge with either LSD or psilocybin after chronic administration of either drug varied between 10 and 30. The decrease in the number of positive responses was, therefore, very great and indicative of a high degree of tolerance and cross tolerance. The large size of the difference, however, skewed the distribution of the data for the group as a whole and made the variance very large, and therefore prevented a statistically significant result.

Since the experiment was not entirely conclusive, and since the failure to reach a completely conclusive result might be due to the small number of subjects, it should be repeated using the same design in the hope that the large number of subjects will reduce the variance. It is also possible that the failure to reach statistical significance was due to a relatively low degree of tolerance to psilocybin. In the latter case, the period of chronic treatment with psilocybin should be extended and, perhaps the psilocybin dosage should be increased to 200 mcg/kg.

Although not entirely conclusive, the results are sufficiently suggestive to support rather strongly the notion that LSD and psilocybin induce a psychosis by some common biochemical or physiological mechanism.

SUMMARY

1. Cross tolerance between LSD-25 and psilocybin was tested by "challenging" 5 men with 150 mcg/kg of psilocybin after they had received LSD-25 once daily for 7-8 days (25 mcg/kg increasing to 150 mcg/kg).

2. A high degree of "direct" tolerance developed to LSD after chronic administration for 7-8 days. The development of "direct" tolerance to psilocybin was not proved conclusively, although the results are strongly suggestive that partial tolerance to psilocybin was present.

3. Responses to psilocybin after chronic administration of LSD are highly suggestive of cross tolerance between LSD and psilocybin. Diminution in the responses to LSD after chronic treatment with psilocybin, while suggestive, were not sufficiently great to establish conclusively the presence of cross tolerance between psilocybin and LSD.

4. The experiment should be extended to include at least 5 other subjects and, if the results are still inconclusive, LSD and psilocybin should be extended for a longer period of time and higher doses given.

REFERENCES

EDWARDS, A. L.: Statistical Analysis for Students in Psychology and Education, 1. New York: Rhinehart and Co., 1946.

HOFMANN, A. R., HEIM, R., BRACK, A., and KOBEL, H.: Psilocybin, ein psychotroper Wirkstaff and dem Mexikanichen Rauschpilz, Psilocybe Mexicana Heim. Experientia (Basel) 14: 107 (1958a).

HOFMANN, A., FREY, A., OTT, H., PETRZILKA, H., and TROXLER, F.: Konstitutionsaufklärung und Synthese von Psilocybin. Experientia (Basel) 14: 397 (1958b).

ISBELL, H.: Comparison of the reactions induced by psilocybin and LSD-25 in man. Psychopharmacologia, in press (1959).

ISBELL, H., BELLEVILLE, R. E., FRASER, H. F., WIKLER, A. and LOGAN, C. R.: Studies on lysergic acid diethylamide (LSD-25). I. Effects in former morphine addicts and development of tolerance during chronic intoxication. A.M.A. Arch. Neurol. & Psychiat., 76: 468-478 (1956).

ISBELL, H., LOGAN, C. R., and MINER, E. J.: Studies on lysergic acid diethylamide (LSD-25). III. Attempts to attenuate the LSD-reaction in man by pretreatment with neurohumoral blocking agents. A.M.A. Arch. Neurol. & Psychiat., 81: 20-27 (1959).

WASSON, V. P. and WASSON, R. G.: Mushrooms, Russia and History. New York: Pantheon Books, 1957.

WINTER, C. A. and FLATAKER, L.: Studies on Heptazone (6-Morpholino-4,4-diphenyl-3-heptanone hydrochloride) in comparison with other analgesic agents. J. Pharmacol. & Exper. Therap., 98: 305-317 (March) 1950.

FOOTNOTE 1.

Footnote 1. We are indebted to Drs. R. Bircher and C. Henze of Sandoz Pharmaceuticals, Hanover, N. J. for supplies of psilocybin and LSD.

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Table 1.

Experimental Design for Testing Cross-Tolerance
Between LSD-25 and Psilocybin (V-C-5).

Period	Day of Experiment	Subjects X[1]	Subjects Y[2]	Remarks
Control #1	1, 2 or 3 4, 5 or 6 8, 9, 10 or 11	LSD, 1.5 mcg/kg Placebo V-C-5, 150 mcg/kg	V-C-5, 150 mcg/kg Placebo LSD, 1.5 mcg/kg	To obtain basal data Order of tests randomized At least five days between tests with LSD and V-C-5
Chronic Adminis-tration #1	12 or 13 to 19 or 20 20 or 21 21 or 22	LSD, increasing to 1.5 mcg/kg LSD, 1.5 mcg/kg V-C-5, 150 mcg/kg	V-C-5, increasing to 150 mcg/kg V-C-5, 150 mcg/kg LSD, 1.5 mcg/kg	To develop tolerance Test of "direct" tolerance Test of "cross" tolerance
Placebo or Withdrawal Period	22 or 23 to 29 or 31	Placebo	Placebo	To lose tolerance
Control #2	30, 31, or 32 33, 34, 35, 36, 37, 38 or 39	Placebo V-C-5, 150 mcg/kg LSD, 1.5 mcg/kg	LSD Placebo V-C-5, 150 mcg/kg	To prove loss of tolerance To replicate basal data Order randomized at least five days between tests with LSD and psilocybin.
Chronic Adminis-tration #2	39 or 40 to 46 or 47 47 or 48 49 or 50	V-C-5, increasing to 150 mcg/kg V-C-5, 150 mcg/kg LSD, 1.5 mcg/kg	LSD, increasing to 1.5 mcg/kg LSD, 1.5 mcg/kg V-C-5, 150 mcg/kg	"Cross-Over" To develop tolerance Test "direct" tolerance Test "cross" tolerance

1. Subjects "X" first received LSD chronically.

2. Subjects "Y" received psilocybin first.

Table 2.

Differences in Responses to Placebo, 1.5 mcg/kg of LSD-25, and 150 mcg/kg of Psilocybin on First and Second Controls.

MEASURE	DRUG		
	Placebo	LSD-25	Psilocybin
Temperature	+ 1.4 ± 0.93	- 0.2 ± 1.1	+ 0.7 ± 0.57
Pulse Rate	-10.8 ± 13.1	-24.8 ± 28.2	-16.1 ± 13.1
Blood Pressure	-22.2 ± 25	-16.6 ± 22	-20.3 ± 9.1
Pupillary Size	+ 0.2 ± 2.3	- 0.02± 1.3	- 0.3 ± 1.32
Kneejerk	- 3.6 ± 9.3	-25.9 ± 21	+25.5 ± 16.5
Responses on Questionnaire	0	- 4 ± 31	- 8.8 ± 13.8
Clinical Grade	0	- 0.2 ± 0.37	0

Figures represent the mean differences ± standard errors of the differences between measurements on the first and second controls in 5 subjects. None of the differences are significant.

+, Indicates that the average measurement was increased on the second control;

-, Indicates that it decreased.

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Table 3.

Differences Between Control Response to LSD-25 and Psilocybin, and Responses after Chronic Administration of Either Drug.

	AFTER LSD CHRONICALLY		AFTER PSILOCYBIN CHRONICALLY	
MEASURE	LSD ("Direct" Tolerance)	Psilocybin ("Cross" Tolerance)	LSD ("Cross" Tolerance)	Psilocybin ("Cross" Tolerance)
Temperature	- 1.4 ± 0.74	- 1.1 ± 0.99	- 0.8 ± 0.93	- 0.7 ± 0.3
Pulse Rate	- 42.0^{x} ± 14.0	-12.5 ± 18.3	-15.8 ± 18.7	-33.2^{x} ± 11.2
Blood Pressure	-108.5^{xx} ± 17.1	-33.4^{x} ± 9.01	-42.7^{x} ± 13.7	-35.1 ± 10.0
Pupillary Size	- 14.0^{xx} ± 2.4	- 8.24^{x} ± 2.2	- 3.1 ± 2.37	- 2.0 ± 1.2
Kneejerk	- 28.7 ± 15.4	-42.7^{xx} ± 8.6	- 6.9 ± 6.7	-30.8^{xx} ± 7.2
Responses on Questionnaire	- 77.2 ± 35.5	-22.4 ± 10.3	-67.8 ± 38.6	-18.0 ± 15.6
Clinical Grade	- 2.3^{xx} ± 0.46	- 1.5^{x} ± 0.45	- 1.8^{x} ± 0.5	- 1.1^{xx} ± 0.23

Figures represent the mean differences ± standard errors between the average of the two controls and the values found when the patient was tested with LSD or psilocybin after receiving either drug chronically.

-, Indicates a decrease in the response after chronic drug administration ("tolerance") as compared with control (nontolerant).

xx, Difference significant at 1% level ($P < 0.01$)

x, Difference significant at 5% level ($P < 0.05$)